J Med Biochem 2010; 29 (4) DOI: 10.2478/v10011-010-0029-4

**UDK 577.1 : 61** **ISSN 1452-8258**

*J Med Biochem 29: 245–253, 2010*

*Review article*
*Pregledni članak*

# MEASURING THYROGLOBULIN CONCENTRATIONS IN PATIENTS WITH DIFFERENTIATED THYROID CARCINOMA

## MERENJE KONCENTRACIJE TIREOGLOBULINA KOD PACIJENATA SA DIFERENTOVANIM KARCINOMIMA ŠTITASTE ŽLEZDE

*Svetlana Savin[1], Dubravka Cvejić[1], Ljiljana Mijatović[2], Snežana Živančević Simonović[2]*

*[1]Institute for the Application of Nuclear Energy – INEP, University of Belgrade, Zemun-Belgrade, Serbia*
*[2]Faculty of Medicine, University of Kragujevac, Kragujevac, Serbia*

**Summary:** Thyroid carcinomas are the most common malignant endocrine tumors. Thyroglobulin (Tg), a specific thyroid protein, is the most important tumor marker in thyroid oncology. After total thyroidectomy or radioiodine therapy, detectable or increasing serum Tg levels in patients with differentiated thyroid carcinoma indicate persistence of active thyroid tissue or cancer recurrence. Serum Tg concentration primarily reflects three variables: the mass of differentiated thyroid tissue present; the degree of thyrotropin receptor stimulation and the intrinsic ability of the tumor to synthesize and secrete Tg. Measurement of serum Tg by current immunometric (IMA) and radioimmunological (RIA) assays encounters some methodological problems which can diminish its clinical importance. Discrepancy between the results for Tg using different methods may be caused by: different reference materials, specific properties of the primary and secondary antibodies for antigenic determinants on Tg and diverse binding affinities of these epitopes, together with interference by serum factors (usually antibodies to Tg (TgAb)) with the primary and secondary Tg antibodies from the diagnostic set. In the presence of endogenous TgAb, Tg values measured by immunoradiometric assay (IRMA) and similar assays are usually lower than the real concentrations, while in RIA apparently lower or higher results can be obtained. Falsely low values may lead to delay in necessary treatment, while an inappropriately high Tg value can cause patient anxiety and unnecessary scans. Despite current methodological limitations, serum Tg measurement is a useful test for determining worsening disease and monitoring the effects of therapy in patients who have undergone surgery for differentiated thyroid carcinoma.

**Keywords:** antithyroglobulin autoantibodies, differentiated thyroid carcinoma, immunometric assay, thyroglobulin

*Address for correspondence:*

Dr Svetlana Savin
Institute for the Application of Nuclear Energy – INEP
11080 Zemun – Belgrade, Banatska 31b, Serbia
Tel. +381 11 3169058
Fax: +381 11 2618724
e-mail: ssavin@inep.co.rs

**Kratak sadržaj:** Tiroidni karcinomi su najčešći maligni endokrini tumori. Tireoglobulin (Tg), specifični protein štitaste žlezde, najvažniji je tumorski marker u tireoidnoj onkologiji. Kod pacijenata sa diferentovanim karcinomima tireoideje, nakon operativnog lečenja, koncentracija Tg određuje se radi otkrivanja rezidualnog tumorskog tkiva ili postojanja lokalnih, odnosno udaljenih metastaza. Na koncentraciju Tg u serumu utiču: masa prisutnog tireoidnog tkiva (benignog ili malignog), intenzitet stimulacije receptora za tireostimulišući hormon (TSH) i sposobnost tumorskih ćelija da sintetišu i luče Tg. Savremene metode, imunometrijske (IMA) i radioimunološke (RIA), kojima se određuje koncentracija Tg u serumu ispitanika, imaju određena ograničenja koja mogu da umanje klinički značaj dobijenih rezultata. Usled metodoloških razlika, koncentracije Tg u istim uzorcima seruma, izmerene različitim testovima, mogu se razlikovati. Faktori koji mogu prouzrokovati razlike u izmerenim koncentracijama Tg su brojni: različiti referentni materijali, razlike u specifičnosti primarnih i sekundarnih antitela za antigenske determinante Tg, različit afinitet vezivanja tih antitela za epitope Tg, i interferencija serumskih faktora. Princip testa, kao i eventualno prisustvo TgAt u serumima ispitanika, može uticati na izmerenu koncentraciju Tg. Svako odstupanje izmerenih koncentracija Tg od stvarnih vrednosti može imati ozbiljne posledice: lažno niske vrednosti Tg mogu odložiti neophodni tretman pacijenata, dok lažno povećane vrednosti Tg mogu prouzrokovati nepotrebni stres, ili čak tretman pacijenata. I pored ograničenih mogućnosti savremenih metoda, određivanje koncentracije Tg u serumu pacijenata operisanih od diferentovanog tiroidnog karcinoma je koristan test za otkrivanje pogoršanja bolesti i za praćenje efekata terapije.

**Ključne reči:** diferentovani tireoidni karcinom, imunometrijski test, tireoglobulin, tireoglobulinska antitela

## Introduction

Thyroid carcinomas are the most common malignant tumors of the endocrine system and their incidence is on the rise (1). Most thyroid carcinomas are differentiated tumors: 88% papillary and 8% follicular (1). Differentiated thyroid carcinoma (DTC) can appear at any time of life with peaks in the third and sixth decade and a median at 44 years (2). Epidemiological data indicating an increase in the number of thyroid carcinomas can partially result from the application of advanced diagnostic methods that can detect micropapillary carcinomas (smaller than 1 cm) or more detailed research of autopsy material which showed a 5–24% incidence of thyroid carcinoma (2).

If thyroid carcinoma is diagnosed in a patient, the physician may decide on operative treatment (with or without application of an ablative dose of radioactive iodine – ($I^{131}$) (3, 4) or clinical follow-up depending on the tumor size and other characteristics (5). Most (90%) thyroid tumors are smaller than 2 cm in diameter and are considered low risk, so less aggressive treatment and monitoring are applied, in accordance with current protocols (3, 4).

In disease diagnostics and monitoring of patients with thyroid carcinoma, determining the serum concentration of thyroglobulin (Tg) is very important. Tg is a glycoprotein with a molecular weight of 660 kDa, synthesized exclusively by thyroid follicular cells – thyrocytes. The fact that the thyroid gland is the only organ synthesizing Tg (6) makes this molecule a marker of differentiated thyroid carcinoma relapse or the occurrence of metastasis in the postoperative period, if the thyroid gland tissue was completely removed by a surgical procedure with eventual use of an ablative dose of radioactive iodine ($I^{131}$). Namely, there is currently no method that can establish whether circulating Tg originates from normal or malignant tissue.

However, due to malignant transformation of thyroid follicular cells, the structure of Tg (especially the carbohydrate component) can be changed (7) together with alterations in the secretion mechanism (8). Epitope mapping on the Tg molecule has shown the existence of six different antigenic regions to which different thyroglobulin-specific antibodies (TgAb) can bind (9, 10), most probably due to differences in Tg molecules secreted from malignantly changed thyroid cells (9, 11–13).

The Tg concentration in serum is a reflection of: thyroid tissue mass presence, disruption of thyroid follicle integrity and degree of thyrostimulating hormone (TSH) receptor stimulation. For newly diagnosed and treated patients with DTC, where the thyroid tissue has not been completely removed, there are combinations of normal remnant and/or tumor tissue, though one must have in mind that not all thyroid tumors can synthesize and secrete Tg (14, 15). Injuries to the thyroid tissue, i.e. disruption of thyroid follicle integrity (fine-needle aspiration biopsy, surgery, radioactive iodine therapy or inflammation) can lead to the release of Tg from follicles where it is stored. Finally, besides the secretion of thyroid hormones, stimulation of TSH receptors releases Tg (endogenous TSH, recombinant human TSH (rhTSH), human chorionic gonadotropin in pregnancy or antibodies that stimulate the TSH receptor in Graves' disease) (15). For patients with differentiated thyroid carcinoma the Tg concentration in sera is determined before and after surgery before applying the ablative dose of radioactive iodine, and also during regular checkups after therapy is completed.

Even though Tg is mainly determined in sera, it can be measured in the needle washout after aspirative biopsy of lymph nodes suspected of DTC metastasis.

## Preoperative Tg measurement

The level of increase of Tg concentration prior to thyroidectomy has no predictive value, although it has been shown that patients with malignant tumors have (on average) higher concentrations of Tg in serum (if it is calculated in relation to the node size) in comparison with patients with benign nodules (18, 19). If biopsy has enabled detection of malignant tissue, high levels of Tg before surgery indicate that the tumor cells produced Tg. Approximately two thirds of patients diagnosed with DTC have high Tg levels in sera prior to surgery (>40 ng/mL). For these patients measuring Tg levels after surgery will be a useful, specific marker for the continuation or worsening of the disease. The sensitivity of Tg measurements after surgery will be higher if the tumor is relatively small (2 cm or smaller) and the Tg value before surgery high. If the Tg value is low before surgery, a non-detectable Tg value after surgery is not a convincing confirmation of the absence of tumor tissue, as it is not clear whether the tumor produced Tg or not (20, 21). For such patients a detectable level of Tg after surgery might indicate the presence of a large tumor mass or the appearance of metastases.

## Measuring Tg after surgery

No detectable Tg in the sera of patients from whom the thyroid gland has been completely removed is usually a confirmation of success of the surgical treatment. However, after surgery for thyroid carcinoma, detectable serum Tg in some patients may originate from a small amount of normal thyroid tissue that has not been removed (near total thyroidectomy). Thus, detectable Tg does not definitely indicate the presence of carcinoma, as it can also

originate from normal (benign) thyroid gland tissue. In such cases patients are given an ablative dose of radioactive iodine ($^{131}I$) to destroy the remaining thyroid tissue. After this tissue is eliminated (that might or might not be malignant), serum concentrations of Tg should be reduced, which will enable higher sensitivity of later measurements of Tg as a marker of relapse or metastasis.

In patients with DTC (papillary or follicular), Tg, which has an average half life of about 65 hours, completely disappears from the circulation within a month after total thyroidectomy (22). Only after this period can one measure the Tg concentration in serum in order to evaluate the disease course, i.e. success of the surgical treatment of thyroid carcinoma.

Determining the concentration of serum Tg as a specific tumor marker of thyroid tissue has eased the postoperative follow-up of patients with thyroid carcinoma and enabled earlier discovery of residual tumor tissue or relapses. Measuring Tg can also be useful in patients where total thyroidectomy was not performed, but interpretation of the results is harder (23, 24). Independently of the type of surgery, it is important to monitor changes in Tg values at certain time intervals (3–6 months).

## Measuring Tg during substitution therapy with thyroid hormones

After surgical treatment of patients with differentiated thyroid carcinoma and removal of the tumor together with healthy functional thyroid tissue, the patients are left without endogenous secretion of thyroid hormones. This slowly leads to clinical hypothyrosis. Due to the lack of thyroid hormones and their inhibitory effect on TSH secretion, TSH secretion increases in these patients, followed by elevated TSH levels in serum. Besides stimulating the thyroid gland to produce thyroid hormones, it has been shown that TSH releases Tg and increases its concentration in the circulation (25, 26) and it is believed to stimulate the growth of most thyroid tumors. During introduction of substitution therapy with thyroid hormones (most often levothyroxine), the dose is increased until suppression of TSH is achieved. If during thyroid hormone therapy TSH levels are suppressed and stable, an increase of Tg concentration in serum (at regular patient checkups) will best reflect changes in tumor size. As there are no reference values for serum concentrations of Tg in patients with DTC after surgery, the relation between Tg levels in serum and thyroid tissue mass can indicate the existence of a tumor and its size. Namely, one gram of normal thyroid tissue releases in the circulation approximately 1 ng/mL Tg, if the TSH level in serum is within reference values (0.4–4.0 IU/L), or 0.5 ng/mL Tg if the TSH level in serum is suppressed below 0.1 IU/L. As the remaining thyroid tissue after total thyroidectomy should not be larger than 2 g, it is expected that the Tg concentration in such a patient's serum should be lower than 2 ng/mL. Some data indicate that patients with high Tg levels after total thyroidectomy almost always have residual thyroid carcinoma (27, 28) or relapses will appear (29).

## Measuring Tg after stimulation with TSH

The most sensitive method for determining residual thyroid carcinoma is measuring Tg concentrations in serum after stimulation with endogenous or exogenous TSH. The term »non-detectable« Tg value refers to disease-free patient status, but it depends on the conditions under which samples are taken for analysis and also test characteristics. Several studies have shown that Tg concentrations will increase after TSH stimulation in 20–25% of patients with undetectable serum Tg (during substitution therapy and TSH suppression) (30–32). Before scintigraphy, patients are taken off thyroid hormone therapy to induce hypothyrosis, until TSH concentrations increase above 25 mIU/L. This increase in TSH will stimulate the release of Tg, when tumor and/or normal thyroid tissue is present and increase accumulation of radioactive iodine in the thyroid gland. Thus, stimulation with TSH increases the test sensitivity for Tg in determining tumor relapses and metastasis (33, 34) and is at the same time an indicator of tumor sensitivity to TSH stimulation. After stimulation with TSH well-differentiated thyroid tumors (and also the remaining normal thyroid tissue) give a tenfold increase in serum Tg concentrations, while the increase of Tg level is usually less than threefold with less differentiated carcinomas. Factors that also influence the elevation of Tg serum concentrations are the duration and level of increased TSH in serum. Patients from whom levothyroxine therapy is withdrawn for several weeks do not bear hypothyrosis easily, so instead of withdrawing the substitution therapy and stimulating endogenous TSH, an alternative method is now used. This includes intramuscular application of recombinant human TSH (rhTSH, Thyrogen), a few days before determining Tg concentrations. According to Mazzaferri et al. (30) both approaches have equivalent sensitivity, though other authors (35, 36) consider that suspending substitution therapy gives higher sensitivity for detection of residual thyroid carcinoma. Current recommendations of the American Thyroid Association are that 6 months after ablation Tg concentrations should be measured after suspending levothyroxine therapy (due to better sensitivity in detecting recurrent or persistent disease), but if Tg values are obtained pointing to a low risk, then further monitoring can be done using rhTSH (3).

## Methodological problems in determining Tg

Measuring Tg in serum is associated with a series of methodological problems that can reduce its clinical significance (37). Nevertheless, there are many commercial diagnostic kits for its determination. According to the detection principle (competitive binding of labelled and unlabelled Tg for a limited number of TgAb or Tg in a sandwich between labelled and unlabelled antibodies), they can be categorized into two groups: radioimmunoassay (RIA) and immunoradiometric assay (IRMA) or broader (IMA) tests. The test principle, its sensitivity and also the eventual presence of serum factors: thyroglobulin antibodies (TgAb) or heterophilic anti-mouse antibodies (HAMA) in patient serum (38, 11), can influence the measured Tg concentrations. Thus, determining Tg in the same serum sample in different laboratories can give different values. Factors causing differences in the serum concentrations of Tg obtained using different tests are numerous: different reference materials, non-harmonized standards for the same reference material, different specificity and also the affinity of primary and secondary antibodies for Tg epitopes, interference of serum factors, primarily thyroglobulin autoantibodies (39–41) with primary and secondary TgAb in the diagnostic kits (42–45). These differences between methods do not allow comparison of results and practically prevent interpretation of changes in Tg levels noted using different methods, such as changes in tumor size or the appearance of metastatic tissue. Therefore, in order to be able to compare the results of control measurements of Tg concentrations in patient serum, Tg measurements must be done using the same method and, if possible, in the same laboratory.

## Influence of test sensitivity on monitoring a patient with DTC

The sensitivity of Tg tests is important for the detection of small DTC. If a test cannot detect Tg in individuals with an intact thyroid gland, then such a test has insufficient (suboptimal) sensitivity for monitoring patients with DTC after thyroidectomy. In principle, IMA tests are more sensitive than RIA tests. Modern guidelines, instead of the term »ultrasensitive«, have defined standard protocols for measuring Tg test sensitivity. Functional sensitivity is defined as the thyroglobulin concentration that can be measured with a 20% coefficient of variation under clinically relevant conditions. Namely, assays of TgAb-negative human sera are repeated over at least a 6–12 month period using at least two lots of reagents and calibrators (46). Conventional tests for measuring Tg have a functional sensitivity of 1 μg/L, while for newer generation tests it is lower than or equal to 0.1 μg/L. Advocates for the application of these tests consider that this will eliminate the need for expensive rhTSH stimulation tests.

There is no agreement concerning Tg cut-off values for detection of a relapse or recurrent disease in patients with DTC. Current guides recommend values of 2 μg/L after stimulation (endogenous or rhTSH) as a risk factor for DTC (3), but instead of a fixed cut-off value the method of monitoring the trend of measured concentrations of basal Tg (without stimulation) can be used (47). It is a good practice in some laboratories to freeze and store sera after determining Tg, so the samples can be used again in parallel with the next sample from the same patient. Results obtained in this way can confirm that changes in Tg levels are the result of alterations in tumor mass and not due to differences in test performance. This approach assists the doctor to conclude with greater certainty whether the disease has worsened or not.

## Reference Tg

Due to differences between methods, a project has started at the European level, supported by the Committee for Reference Materials of the European Union Commission, for isolation and characterization of reference Tg. The result of this project is a certified reference material for thyroglobulin – CRM-457 (48, 49). Most tests are now calibrated, directly or indirectly, according to this standard. However, regardless of standardization according to CRM 457, there is still great variability between different tests for determining Tg. This could be a reflection of diverse specificities in individual tests for Tg isoforms (37, 50–54) and interference by serum factors, primarily autoantibodies (TgAb), with reagent antibodies in the assays. When six diagnostic kits standardized according to CRM 457 (2 RIA and 4 IRMA) were analyzed, almost twofold differences for Tg concentrations in serum without TgAb were obtained (15). The coefficient of variation for these six tests was 37%, which is more than double the biological variability in healthy euthyroid individuals (55) or in individuals with DTC and suppressed TSH (15).

The results obtained using IMA techniques varied more than for RIA (37), most probably because polyclonal antibodies with wider epitopic specificities are used in RIA and they can detect tumor-modified Tg isoforms better than IMA techniques that employ monoclonal antibodies with limited epitopic specificities. This can be significant, as the posttranslatory changes of Tg are altered in tumor cells, leading to conformation changes in secreted Tg (53). Due to the type of conformation of Tg epitopes, it is not surprising that larger differences have been shown between individual IRMA methods than for RIA tests.

## Interference with serum factors

Interference with antibodies against human immunoglobulin (HAMA) occurs in about 3% of sera sent for determination of Tg concentration (56). For

IMA tests HAMA mainly produces a false increase (56) and more rarely false negative Tg values (57). One should consider interference caused by HAMA if the measured Tg concentration does not correspond to the patient's clinical status and, if possible, Tg in the presence of HAMA should be measured by RIA.

According to data in the literature, increased concentrations of TgAb are present in 20–30% of patients with DTC (58, 59). Successful removal of the tumor leads to a decline in TgAb concentration and antibodies disappear from the circulation after about 3 years (60). Disappearance of circulating TgAb indicates a positive outcome, i.e. an insignificant risk for the occurrence of relapse or metastasis in patients with DTC. On the other hand, some studies have indicated a higher incidence of residual thyroid carcinoma in patients with high TgAb values compared to patients with low or non-detectable TgAb values (61, 62).

The presence of Tg-specific autoantibodies influences the measured Tg concentration in most assays and can depend on their affinity and capacity on the one hand, and the properties of reagents in the tests for determining Tg concentrations on the other hand (37, 41, 43).

Depending on the type of method used, samples with TgAb can give inappropriate higher or lower Tg values. It has generally been accepted that RIA tests are less sensitive to the presence of TgAb than IRMA tests (63). Measured serum Tg concentrations using RIA tests can sometimes be lower, but are often higher than the true values (39, 40). Nowadays, many laboratories employ immunometric tests, as they have certain technical advantages such as shorter incubation time and process automation (37, 41, 46).

The measured values of Tg using IRMA (or similar) tests are usually lower than the actual levels (63), even though TgAb concentrations may be very low, even below positive limits (59, 64, 65). It seems that values for Tg measured by RIA tests correspond more closely to physiological values, as both free Tg and Tg complexed with TgAb are detected (15). The principle of RIA tests is competitive binding by polyclonal antibodies, which have greater possibilities of recognizing epitopes exposed on Tg molecules bound to TgAb (9, 11–13). The method of disrupting binding of the TgAb-Tg complex to antibody in IMA tests could be twofold: stearic inhibition or masking of the epitope that recognizes the monoclonal antibody in the diagnostic kit, but there are no experimental data for this mechanistic concept (9, 11, 66).

In the presence of TgAb, the measured values of Tg should be cautiously interpreted regardless of the applied test. Falsely low values for serum Tg concentration can postpone the required patient treatment. In contrast, falsely increased Tg values measured using RIA tests can initiate additional screening or treatment that is an unnecessary stress for the patient.

For now there is no expert agreement on the question of selection of the method best suited for Tg measurement in sera where, using a sensitive enough method, the presence of TgAb has been shown. With RIA methods there is less interference with Tg specific autoantibodies and some laboratories in the USA consider that they provide more clinically accurate values, and so give the advantage to RIA methods for measuring serum Tg (37). Moreover, it is considered that IMA methods should not be used when TgAb is present in patient serum, as falsely low Tg values are a greater problem than falsely high ones. For example, a falsely low Tg value obtained due to the interference of endogenous TgAb can lead to postponement of necessary treatment, while a falsely high value of Tg obtained in the presence of TgAb in the patient's serum will only direct the doctor's attention to this patient.

## Identification of the influence of TgAb

As the presence of TgAb in the patient's serum can significantly influence the measured Tg value, it is important to use a precise and sensitive method for determining TgAb. Unfortunately, the results obtained using different methods for determining TgAb concentrations differ even more than the results for Tg measurements. Thus, one assay can show that there is an increased concentration of anti-thyroglobulin autoantibodies in a certain patient's serum, while another detects no TgAb in the same serum (67, 68).

Besides measuring the presence of TgAb with an adequately sensitive method, the presence of these autoantibodies in a patient's serum can be established by adding a known amount of Tg to serum samples (»recovery test«) and also by comparison of the degree of agreement of Tg results using tests based on different analytical principles (IRMA/RIA). If TgAb is present in the samples, the values for Tg measured using RIA are usually higher than the values obtained by IRMA (34, 59). Thus, if the results obtained using IRMA and RIA methods show good agreement, there is a large possibility that the finding is acceptable for clinical diagnostics. This approach enables identification of samples where interactions occur between autoantibodies and Tg specific reagent antibodies in the test for determining Tg, but it does not direct the doctor's actions, as it cannot answer the question of how the different values obtained relate to the real concentration of Tg in the sample. To evaluate the influence of TgAb on Tg concentrations measured by RIA and IRMA tests, it is necessary to do a »recovery test« for individual samples where disagreement between measured Tg

values has been shown. Though not ideal, the »recovery test« does indicate whether the Tg value measured using a certain method is lower or higher than the real one.

Of course, the serum concentration of Tg is only one parameter in the postoperative monitoring of patients with differentiated thyroid carcinoma, and if there is a difference in the results obtained using methodologically different tests, a clinical practitioner should take into account that neither result is true and undertake additional clinical investigations.

## Measuring TgAb in serum

It is necessary to determine TgAb concentrations in every serum sample where Tg is measured, for two reasons: first, the patient's TgAb status can change from positive to negative or vice versa, and secondly, to follow the trend of TgAb concentration change over a long time period in order to evaluate how the tumor reacts to therapy (for example, when applying ablative $^{131}$I doses). Serial determination of TgAb is sometimes recommended as a surrogate tumor marker for DTC (59, 60, 69). The TgAb concentration strives to follow the Tg trend (specific antigen) that is recognized by the immune system. When TgAb is present in the serum of a patient with DTC before surgery and the antigen source is removed, TgAb cannot be detected after 3 years (60, 70) and its concentration is reduced by 50% after 6 months, in accordance with the half-life of circulating endogenous immunoglobulin (49, 71). However, the concentration of TgAb can increase (or become positive in patients negative before the ablative dose) 6 months after applying doses of $^{131}$I in response to the release of Tg from destroyed thyroid tissue (72). Interpretation of TgAb concentrations as a surrogate tumor marker is possible if tests from the same manufacturer are used during the monitoring of a patient, if possible in the same laboratory. Despite standardization according to the international reference material, MRC 65/93, commercial tests for TgAb have different sensitivities, specificities and measured absolute values (45, 46, 59, 69, 73), most probably due to differences in test specificities for different conformation epitopes of TgAb (69, 71, 74). A declining trend for TgAb over a long time period is a good sign of treatment efficacy (surgery/radioiodine ablation). In contrast, if TgAb levels increase with time, this can be an early sign of worsening disease (56, 61, 75). Thus, measuring TgAb concentrations in serum may serve as a surrogate marker for evaluating disease progress (besides measuring Tg concentrations). However, one study (76) based on data obtained by monitoring changes of TgAb levels in the serum of patients who had had surgery for DTC, disputes the value of Tg-specific antibodies as a surrogate tumor marker. Namely, during a three year follow-up no difference in TgAb concentrations was detected between patient groups with or without a relapse of thyroid carcinoma.

## Conclusion

To conclude, we can say that measuring Tg in the serum of patients who had undergone surgery for differentiated thyroid carcinoma is a useful test for determining worsening disease and monitoring the effects of therapy. Patients should be continually monitored using one selected method and if possible in the same laboratory. In order to obtain optimal results it is necessary to understand the limitations of modern methods used in clinical practice and the pathophysiology of Tg secretion.

*Acknowledgements:* This research was supported by the Ministry of Science and Technological Development of the Republic of Serbia (project 143039 and 145039).

## Conflict of interest statement

The authors stated that there are no conflicts of interest regarding the publication of this article.

## References

1. Davies I, Welch HG. Increasing incidence of thyroid cancer in the United States 1973–2002. JAMA 2006; 293: 2164–7.
2. Kovacs GL, Gonda G, Vadasz G, Ludmany G, Uhrin E, Gorombey K, et al. Epidemiology of thyroid microcarcinoma found in autopsy series conducted in areas of different iodine intake. Thyroid 2005; 15: 152–7.
3. Cooper DS, Doherty GM, Haugen BR, Kloos RT, Lee SL, Mandel SJ, et al. Management guidelines for patients with thyroid nodules and differentiated thyroid cancer. The American Thyroid Association Guidelines Taskforce. Thyroid 2006; 16: 109–42.
4. Pacini F, Schlumberger M, Dralle H, Elisei R, Smit JW, Wiersinga W. European consensus for the management of patients with differentiated thyroid carcinoma of the follicular epithelium. Eur J Endocrinol 2006; 154: 787–803.
5. Ito Y, Uruno T, Nakano K, Fukushima M, Kihara M, Higashiyama T, et al. An observation trial without surgical treatment in patients with papillary microcarcinoma of thyroid. Thyroid 2003; 13: 381–7.
6. Van Herle AJ, Vassart G, Dumont JE. Control of thyroglobulin synthesis and secretion. (First of two parts). N Engl J Med 1979; 301: 239–49.
7. Sinadinović J, Cvejić D, Savin S, Jancić-Zuguricas M, Mićić JV. Altered terminal glycosylation of thyroglobulin

in papillary thyroid carcinoma. Exp Clin Endocrinol 1992; 100: 124–8.

8. Sinadinović J, Cvejić D, Savin S, Mićić JV, Jančić-Zguricas M. Enhanced acid protease activity of lysosomes from papillary thyroid carcinoma. Cancer 1989; 63: 1179–82.
9. Okosieme OE, Evans C, Moss L, Parkes AB, Premawardhana LD, Lazarus JH. Thyroglobulin antibody in serum of patients with differentiated thyroid cancer: relationship between epitope specificities and thyroglobulin recovery. Clin Chem 2005; 51: 729–34.
10. Estienne B, McIntosh RS, Ruf J, Asghar MS, Watson PF, Carayon P, et al. Comparative mapping of cloned human and murine antithyroglobulin antibodies: recognition of human antibodies of an immunodominant region. Thyroid 1998; 8: 643–8.
11. Mariotti S, Barbesino G, Caturegli P, Marinó M, Manetti L, Pacini F, et al. Assay of thyroglobulin in serum with thyroglobulin autoantibodies: an unobtainable goal? J Clin Endocrinol Metab 1995; 80: 468–72.
12. Erali M, Bigelow RB, Meikle AW. ELISA for thyroglobulin in serum. Recovery studies to evaluate autoantibody interference and reliability of thyroglobulin values. Clin Chem 1996; 42: 766–70.
13. Trbojević B, Nedeljković-Beleslin B. Importance of hormones and proteins determination in the material obtained by fine-needle aspiration. Journal of Medical Biochemistry 2010; 29: 237–44.
14. Ladenson PW. Optimal laboratory testing for diagnosis and monitoring of thyroid nodules, goiter, and thyroid cancer. Clin Chem 1996; 42: 183–7.
15. Spencer CA, LoPresti JS. Technology insight: measuring thyroglobulin and thyroglobulin autoantibody in patients with differentiated thyroid cancer. Endocrinology and Metabolism 2008; 4: 223–33.
16. Boi F, Baghino G, Atzeni F, Lai ML, Faa G, Mariotti S. The diagnostic value for differentiated thyroid carcinoma metastasis of thyroglobulin (Tg) measurement in washout fluid from fine-needle aspiration biopsy of neck limph nodes is maintained in the presence of circulating anti-Tg antibodies. J Clin Endocrinol Metab 2006; 91: 1364–9.
17. Cunha N, Rodrigues F, Curado F, Ilheu O, Cruz C, Naidenov P, et al. Thyroglobulin detection in fine-needle aspirates of cervical lymph nodes: a technique for the diagnosis of metastatic differentiated thyroid cancer. Eur J Endocrinol 2007; 157: 101–7.
18. Hocevar M, Auersperg M. Role of serum thyroglobulin in the preoperative evaluation of follicular thyroid tumours. Eur J Surg Oncol 1998; 24: 553–7.
19. Sharma AK, Sarda AK, Chattopadhyay TK, Kapur MM. The role of estimation of the ratio of preoperative serum thyroglobulin to the thyroid mass in predicting the behaviour of well differentiated thyroid cancers. J Postgrad Med 1996; 42: 39–42.
20. Spencer CA, Wang CC. Thyroglobulin measurement. Techniques, clinical benefits, and pitfalls. Endocrinol Metab Clin North Am 1995; 24: 841–63.
21. Giovanella L, Ceriani L, Ghelfo A, Maffioli M, Keller F. Preoperative undetectable serum thyroglobulin in differentiated thyroid carcinoma: incidence, causes and management strategy. Clin Endocrinol (OxF) 2007; 67: 547–51.
22. Hocevar M, Auersperg M, Stanovnik L. The dynamics of serum thyroglobulin elimination from the body after thyroid surgery. Eur J Surg Oncol 1997; 23: 208–10.
23. Van Wyngaarden K, McDougall IR. Is serum thyroglobulin a useful marker for thyroid cancer in patients who have not had ablation of residual thyroid tissue? Thyroid 1997; 7: 343–46.
24. Grünwald F, Menzel C, Fimmers R, Zamora PO, Biersack HJ. Prognostic Value of Thyroglobulin after Thyroidectomy before Ablative Radioiodine Therapy in Thyroid Cancer. J Nucl Med 1996; 37: 1962–4.
25. Herle van AJ, Vassart G, Dumont JE. Control of thyroglobulin synthesis and secretion (second of two parts). N Engl J Med 1979; 301: 307–14.
26. Sinadinović J, Mićić JV, Krainčanić M, Kostić G, Savin S. Korelacija između nivoa Tg u cirkulaciji, funkcije tireoideje i njene strukture. Radiol Iugoslav 1982; 16: 239–43.
27. Lin JD, Huang MJ, Hsu BR, Chao TC, Hsueh C, Liu FH, et al. Significance of postoperative serum thyroglobulin levels in patients with papillary and follicular thyroid carcinomas. J Surg Oncol 2002; 80: 45–51.
28. Ronga G, Filesi M, Ventroni G, Vestri AR, Signore A. Value of the first serum thyroglobulin level after total thyroidectomy for the diagnosis of metastases from differentiated thyroid carcinoma. Eur J Nucl Med 1999; 26: 1448–52.
29. Kim TY, Kim WB, Kim ES, Ryu JS, Yeo JS, Kim SC, et al. Serum thyroglobulin levels at the time of 131I remnant ablation just after thyroidectomy are useful for early prediction of clinical recurrence in low-risk patients with differentiated thyroid carcinoma. J Clin Endocrinol Metab 2005; 90: 1440–5.
30. Mazzaferri EL, Robbins RJ, Spencer CA, Braverman LE, Pacini F, Wartofsky L, et al. A consensus report on the role of serum thyroglobulin as a monitoring method for low-risk patients with papillary thyroid carcinoma. J Clin Endocrinol Metab 2003; 88: 1433–41.
31. Baudin E, Do Cao C, Cailleux AF, Leboulleux S, Travagli JP, Schlumberger M. Positive predictive values of serum thyroglobulin levels, measured during the first year of follow-up after thyroid hormone withdrawal in thyroid cancer patients. J Clin Endocrinol Metab 2003; 88: 1107–11.
32. David A, Blotta A, Rossi R, Zatelli MC, Bondanelli M, Roti E, et al. Clinical value of different responses of serum thyroglobulin to recombinant human thyrotropin in the follow-up of patients with differentiated thyroid carcinoma. Thyroid 2005; 15: 158–64.
33. Haugen BR, Pacini F, Reiners C, Schlumberger M, Ladenson PW, Sherman SI, et al. A comparison of recombinant human thyrotropin and thyroid hormone withdrawal for the detection of thyroid remnant or cancer. J Clin Endocrinol Metab 1999; 84: 3877–85.
34. Spencer CA, LoPresti JS, Fatemi S, Nicoloff JT. Detection of residual and recurrent differentiated thyroid

carcinoma by serum thyroglobulin measurement. Thyroid 1999; 9: 435–41.

35. Pacini F, Molinaro E, Castagna MG, Agate L, Elisei R, Ceccarelli C, et al. Recombinant human thyrotropin-stimulated serum thyroglobulin combined with neck ultrasonography has the highest sensitivity in monitoring differentiated thyroid carcinoma. J Clin Endocrinol Metab 2003; 88: 3668–73.
36. Pellegriti G, Scollo C, Regalbuto C, Attard M, Marozzi P, Vermiglio F, et al. The diagnostic use of the rhTSH/thyroglobulin test in differentiated thyroid cancer patients with persistent disease and low thyroglobulin levels. Clin Endocrinol (Oxf) 2003; 58: 556–61.
37. Spencer CA, Bergoglio LM, Kazarosyan M, Fatemi S, LoPresti JS. Clinical impact of thyroglobulin (Tg) and Tg autoantibody method differences on the management of patients with differentiated thyroid carcinomas. J Clin Endocrinol Metab 2005; 90: 5566–75.
38. Black EG, Hoffenberg R. Should one measure serum thyroglobulin in the presence of anti-thyroglobulin antibodies? Clin Endocrinol 1983; 19: 597–601.
39. Schneider AB, Pervos R. Radioimmunoassay of human thyroglobulin: effect of antithyroglobulin autoantibodies. J Clin Endocrinol Metab 1978; 47: 126–37.
40. Feldt-Rasmussen U, Rasmussen AK. Serum thyroglobulin (Tg) in presence of thyroglobulin autoantibodies (TgAb). Clinical and methodological relevance of the interaction between Tg and TgAb in vitro and in vivo. J Endocrinol Invest 1985; 8: 571–6.
41. Spencer CA, Takeuchi M, Kazarosyan M. Current status and performance goals for serum thyroglobulin assays. Clin Chem 1996; 42: 164–73.
42. Žarković M. Diagnosis of thyroid disease. Principles and problems. Journal of Medical Biochemistry 2010; 29: 231–36.
43. Clark PM, Beckett G. Can we measure serum thyroglobulin? Ann Clin Biochem 2002; 39: 196–202.
44. Stanojević M, Savin S, Cvejić D, Đukić A, Živančević Simonović S. Correlation of thyroglobulin concentrations measured by radioimmunoassay and immunometric assay and the influence of thyroglobulin antibody. J Immunoassay Immunochem 2009; 30: 197–207.
45. Stanojević M, Savin S, Cvejić D, Djukić A, Jeremić M, Živancević-Simonović S. Comparison of the influence of thyroglobulin antibodies on serum thyroglobulin values from two different immunoassays in post surgical differentiated thyroid carcinoma patients. J Clin Lab Anal 2009; 23: 341–6.
46. Baloch Z, Carayon P, Conte-Devolx B, Demers LM, Feldt-Rasmussen U, Henry JF, et al. Guidelines Committee, National Academy of Clinical Biochemistry. Laboratory medicine practice guidelines: laboratory support for the diagnosis and monitoring of thyroid disease. Thyroid 2003; 13: 3–126.
47. Zophel K, Wunderlich G, Smith BR. Serum thyroglobulin measurements with a high sensitivity enzyme-linked immunosorbent assay: is there a clinical benefit in patients with differentiated thyroid carcinoma? Thyroid 2003; 13: 861–5.
48. Feldt-Rasmussen U, Profilis C, Colinet E, Black E, Bornet H, Bourdoux P, et al. Human thyroglobulin reference material (CRM 457). 2nd part: Physicochemical characterization and certification. Ann Biol Clin (Paris) 1996; 54: 343–8.
49. Feldt-Rasmussen U, Schlumberger M. European interlaboratory comparison of serum thyroglobulin measurement. J Endocrinol Invest 1988; 11: 175–81.
50. Spencer CA. Recoveries cannot be used to authenticate thyroglobulin (Tg) measurements when sera contain Tg autoantibodies. Clin Chem 1996; 42: 661–3.
51. Ferrari L, Biancolini D, Seregni E, Aliberti G, Martinetti A, Villano C, et al. Critical aspects of immunoradiometric thyroglobulin assays. Tumori 2003; 89: 537–9.
52. Schultz R, Bethauser H, Stempka L, Heilig B, Moll A, Hufner M. Evidence for immunological differences between circulating and tissue-derived thyroglobulin in men. J Clin Invest 1989; 19: 459–63.
53. Hajduković Lj, Savin S, Čuperlović M, Movsesijan M, Sinadinović J. Karakterizacija 125J–h–tireoglobulina pomoću afinitetne hromatografije na koloni fitohemaglutinin-sefaroze 4B. Med Raz 1988; 27: 27–33.
54. Savin S, Sofronić LJ, Sinadinović J. Occurrence of antithyroxine antibodies in rabbits immunized with iodine-poor human thyroglobulin. Exp Clin Endocrinol 1990; 95: 375–83.
55. Feldt-Rasmussen U, Hyltoft Petersen P, Blaabjerg O, Horder M. Long-term variability in serum thyroglobulin and thyroid related hormones in healthy subjects. Acta endocrinol (Copenh) 1980; 95: 328–34.
56. Preissner CM, O'Kane DJ, Singh RJ, Morris JC, Grebe SKG. Phantoms in the assay tube: heterophile antibody interferences in serum thyroglobulin assays. J Clin Endocrinol Metab 2003; 88: 3069–74.
57. Giovanella L, Ghelfo A. Undetectable serum thyroglobulin due to negative interference of heterophile antibodies in relapsing thyroid carcinoma. Clin Chem 2007; 53: 1871–2.
58. Pacini F, Mariotti S, Formica N, Elisei R. Thyroid autoantibodies in thyroid cancer: incidence and relationship with tumor outcome. Acta Endocrinol 1988; 119: 373–80.
59. Spencer CA, Wang C, Fatemi S, Guttler RB, Takeuchi M, Kazarosyan M. Serum Thyroglobulin Autoantibodies: Prevalence, influence on serum thyroglobulin measurement and prognostic significance in patients with differentiated thyroid carcinoma. J Clin Endocrinol Metab 1998; 83: 1121–7.
60. Chiovato L, Latrofa F, Braverman LE, Pacini F, Capezzone M, Masserini L, et al. Disappearance of humoral autoimmunity after complete removal of thyroid antigens. Ann Intern Med 2003; 139: 346–51.
61. Hjiyiannakis P, Mundy J, Harmer C. Thyroglobulin antibodies in differentiated thyroid cancer. Clin Oncol (R Coll Radiol) 1999; 11: 240–4.
62. Chung JK, Park YJ, Kim TY, So Y, Kim SK, Park DJ, et al. Clinical significance of elevated level of serum antithyroglobulin antibody in patients with differentiated thyroid cancer after thyroid ablation. Clin Endocrinol (Oxf) 2002; 57: 215–21.

63. Spencer CA. J Editorial: Challenges of serum thyroglobulin (Tg) measurement in the presence of Tg autoantibodies. Clin Endocrinol Metab 2004; 89: 3702–4.

64. Cubero JM, Rodriguez-Espinosa J, Gelpi C, Estorch M, Corcoy R. Thyroglobulin autoantibody levels below the cut-off positivity can interfere with thyroglobulin measurement. Thyroid 2003; 13: 659–61.

65. Stanojević M, Petrović M, Đukić A, Savin S, Dimitrijević Lj, Živančević-Simonović S. Uticaj antitireoglobulinskih antitela na koncentraciju tireoglobulina izmerenu imunoradiometrijskim testom. Med časopis 2007; 41: 18–19.

66. Henry M, Malthiery Y, Zanelli E, Charvet B. Epitope mapping of human thyroglobulin: heterogeneous recognition by thyroid pathologic sera. J Immunol 1990; 145: 3692–8.

67. Savin S, Sinadinović J, Mićić JV, Movsesijan M. Radioimunološka metoda (RIA) za određivanje tireoglobulinskih autoantitela u humanom serumu. Radiol Iugoslav 1983; 1: 363–6.

68. Sinadinović J, Sofronić Lj, Prelević G, Čuperlović K, Savin S. Immunochemical method for determination of thyroglobulin autoantibodies. Periodicum Biologorum 1983; 85: 255–6.

69. McLachlan SM, Rapoport B. Why measure thyroglobulin autoantibodies rather than thyroid peroxidase autoantibodies? Thyroid 2004; 14: 510–20.

70. Thomas D, Liakos V, Vassiliou E, Hatzimarkou F, Tsatsoulis A, Kaldrimes P. Possible reasons for different pattern disappearance of thyroglobulin and thyroid peroxidase autoantibodies in patients with differentiated thyroid carcinoma following total thyroidectomy and iodine-131 ablation. J Endocrinol Invest 2007; 30: 173–80.

71. Tumino S, Belfore A. Appearance of antithyroglobulin antibodies as the sole sign of metastatic lymph nodes in patients operated on for papillary thyroid cancer: a case report. Thyroid 2000; 10: 431–3.

72. Feldt-Rasmussen U, Bech K, Date J, Hyltoft Pedersen P, Johansen K, Nistrup Madsen S. Thyroid stimulating antibodies, thyroglobulin antibodies and serum proteins during treatment of Graves disease with radioiodine or propylthiouracil. Allergy 1982; 37: 161–7.

73. Sapin R, D'Herbomez M, Gasser F, Meyer L, Schlinger JL. Increased sensitivity of a new assay for antithyroglobulin antibody detection in patients with autoimmune thyroid disease. Clin Biochem 2003; 36: 611–16.

74. Benvenga S, Burek CL, Talor M, Rose NR, Trimarchi F. Heterogeneity of the thyroglobulin epitopes associated with circulating thyroid hormone autoantibodies in Hashimoto's thyroiditis and non-autoimmune thyroid diseases. J Endocrinol Invest 2002; 25: 977–82.

75. Rubello D, Casara D, Girelli ME, Piccolo M, Busnardo B. Clinical meaning of circulating antithyroglobulin antibodies in differentiated thyroid cancer: a prospective study. J Nucl Med 1992; 33: 1478–80.

76. Quevedo I, Campino C, Rodríguez Portales JA, Arteaga E, López JM, Campusano C, et al. Anti-thyroglobulin antibodies in the follow-up of patients with differentiated thyroid cancer: residual or relapsing disease markers? Rev Med Chil 2002; 130: 167–72.

*Received: April 30, 2010*

*Accepted: June 4, 2010*